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Rhizobium and Phosphate Solubilizing Bacteria Improve the Yield and Phosphorus Uptake in Wheat (*Triticum aestivum*)

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ABSTRACT

Rhizobium and phosphorus (P) solubilizing bacteria are important to plant nutrition. These microbes also play a significant role as plant growth-promoting rhizobacteria (PGPR) in the biofertilization of crops. A study was conducted in order to investigate the effects of a rhizobial strain (Thal 8) and a P solubilizer strain (54RB) in single and dual combination with and without P_2O_5 on wheat in a P deficient natural non-sterilized sandy loam soil. The results of this pot experiment revealed that single and dual inoculation with fertilizer (P_2O_5) significantly increased root and shoot weight, plant height, spike length, grain yield, seed P content, leaf protein and leaf sugar content of the test crop. It is concluded that single and dual inoculation along with P fertilizer is 30-40% better than only P fertilizer for improving grain yield of wheat and dual inoculation without fertilizer (P) improved grain yield up to 20% as compared to P application.

Key Words: Phosphate solubilizing bacteria; P uptake; *Rhizobium*; Wheat yield

INTRODUCTION

Soil-plant-microbe interaction has got much importance in recent decades. Many types of microorganisms are known to inhabit soil, especially rhizosphere and play important role in plant growth and development. Phosphate solubilizing bacteria (PSB) are used as biofertilizer since 1950's (Kudashev, 1956; Krasilinikov, 1957). These microorganisms secrete different types of organic acids e.g., carboxylic acid (Deubel & Merbach, 2005) thus lowering the pH in the rhizosphere (He & Zhu, 1988) and consequently dissociate the bound forms of phosphate like $Ca_3(PO_4)_2$ in calcareous soils.

Use of these microorganisms as environment friendly biofertilizer helps to reduce the much expensive phosphatic fertilizers. Phosphorus biofertilizers could help increase the availability of accumulated phosphate (by solubilization), efficiency of biological nitrogen fixation and increase the availability of Fe, Zn etc., through production of plant growth promoting substances (Kucey *et al*., 1989). Trials with PSB indicated yield increases in rice (Tiwari *et al.,* 1989), maize (Pal, 1999) and other cereals (Afzal *et al.,* 2005; Ozturk *et al*., 2003).

Rhizobium previously well known as a symbiotic N fixer is reported as asymbiotic (associative & endophytic) microorganisms in recent years (Biswas *et al*., 2000a, b). The most inoculation studies have focused on free living diazotrophs, although a few reports indicate r*hizobia* can act as plant growth promoting rhizobacteria (PGPR) (Hoflich *et al*., 1995; Noel *et al*., 1996; Yanni *et al*., 1997). The PGPR influence the crop growth and development by releasing plant growth regulators (Glick & Bashan, 1997; Volpin & Philips, 1998) and improving morphological characteristics of inoculated roots (Biswas, 1998), which favored nutrient uptake (Okon & Kapulnik, 1986). The growth promoting effects of rhizobacteria may include phytohormone production (Chabot *et al*., 1996), N_2 fixation (Urquiaga *et al*., 1992) and more efficient use of nutrients (Chabot *et al*., 1996). Yanni *et al.* (1997) and Biswas (1998) reported increased N uptake in rice plants inoculated with rhizobia.

Combined inoculation with N_2 fixing and phosphate solubilizing bacteria was more effective than single microorganism for providing a more balanced nutrition for plants (Belimov *et al*., 1995). Dual inoculation increased yields in sorghum (Algawadi & Gaur, 1992), barley (Belimov *et al*., 1995), black gram (Tanwar *et al*., 2002), soybean (Abdalla & Omar, 2001) and wheat (Galal, 2003).

Reports regarding co-inoculation of *Rhizobium* and P solubilizing bacteria on wheat are rare, especially in Pakistan very little work has been done on these lines. The objective of present investigation was to explore the effect of single and dual inoculations with N_2-fixing and P-soulbilizing bacterial species on wheat yield and P uptake and to explore the hidden potential of *Rhizobium* as a PGPR for this important non-legume.

MATERIALS AND METHODS

A pot experiment was carried out in experimental area of Quaid-i-Azam University Islamabad, Pakistan, to investigate the effects of single and dual inoculations with N-fixing bacteria (*Rhizobium leguminismarum* Thal-8/SK8) and phosphorus solubilizing bacteria (*Pseudomonas* sp. strain 54RB) on yield and yield components of wheat (*Triticum aestivum*) variety Margalla 99. The bacterial strains, Thal-8 originally isolated from Thal area and 54RB isolated from rice, were obtained from Soil Biology and

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Biochemistry Lab. National Agricultural Research Centre (NARC) Islamabad, Pakistan. Wheat seeds were obtained from Institute of Field Crops (wheat section), NARC Islamabad. Clay pots (10 kg soil capacity) were used for trial. Sandy loam soil having organic matter 0.65%, pH 7.9, P content 8 ppm and NO_3-N 10 ppm collected from farmer field was used. Urea and single super phosphate (SSP) were applied as source of N and P respectively.

Un-sterilized soil was air dried and mixed with sand in 1:1 ratio and filled in pots. Composite soil samples were collected and analyzed (analysis given above). These pots were placed under natural conditions. Urea was mixed in upper soil (0-10 cm) @ 1.8 g pot^{-1} before sowing. Similarly SSP was applied as treatment in respective pots @ 2 g per pot. Eight seeds were sown in each pot. With average minimum temperature of 9^{o}C and average maximum temperature of 25^{o}C, a total rainfall of 430 mm, relative humidity of 80% and day length ranging from 10-13 h, plant growth in the region is restricted to the period between November and May. The experimental design for the study was completely randomized block design with six replicates. Treatments were: T0 control (without inoculation & with N) T1 (P_2O_5), T2 (*Rhizobium* @10 mL broth culture per plant), T3 (PSB @10 mL broth culture per plant), T4 (PSB+*Rhizobium*), T5 (P_2O_5+*Rhizobium*), T6 (P_2O_5+PSB) and T7 (P_2O_5+PSB+*Rhizobium*).

Inoculum for *Rhizobium* was prepared in yeast mannitol (YM) broth (Somasegaran & Hoben, 1985) by inoculating autoclaved YM with *Rhizobia* (1 mL) and placed in shaker at 28^{o}C. Inoculum for PSB was prepared in Pikovskaya medium by the same procedure. Inoculation @10 mL per seedling was done at log phase i.e., about 10^8 cells mL^{-1} at the base of plant. Five plants per pot were kept after thinning and grown until maturity to get final yield.

During the growing period, leaf samples were collected (at 108 days after sowing) for sugar and protein analysis before heading. The analyses were done according to Johnson *et al.* (1966) and Lowry *et al.* (1951) respectively. At maturity, data regarding plants height, spike length, root weight, shoots weight and grain yield were recorded using standard procedures. Soil samples from each treatment were collected and analyzed for phosphorus content according to Soltanpur and Workman (1979). Seed P content was measured according to Cottenie (1980). In the present study, P solubilizing ability of 54RB and Thal-8 was demonstrated based on the qualitative methods (Metha & Nautiyal, 2001) under the laboratory condition. Indole acetic acid (IAA) and gibberellic acid (GA) production for both the strains was also determined using HPLC (Table I). Phosphate solubilization was determined using following formula:

$$SI = \frac{\text{colony diameter + halozone diameter}}{\text{Colony diameter}}$$

Statistical analysis. The data obtained in the study were subjected to analysis of variance using MSTATC, computer software (Bricker, 1991) and means were compared by Duncan's Multiple Range Test (Duncan, 1955). Correlations between growth parameter (s) were also calculated (Bricker, 1991).

RESULTS AND DISCUSSION

In this study generally all the parameters were significantly affected by inoculation treatments over control (Table II). Root weight and seed P content (%), however, were non-significantly affected by dual inoculation without fertilizer P (Table II). The highest shoot weight was obtained in pots, which received *Rhizobium* inoculation along with fertilizer that was statistically similar to pots, which received PSB with fertilizer and dual inoculation (*Rhizobium* + PSB) with fertilizer (Table II). Single inoculation of *Rhizobium* and PSB behaved similar to that of control for root and shoot weight (Table II). Dual inoculation with fertilizer yielded maximum root weight followed by single inoculation of *Rhizobium* with P and single inoculation of PSB along with P. Control and other treatments had similar root weight (Table II). Increased root, shoot weight with dual inoculation have been reported by Höflich *et al*. (1994) in barley, Yanni *et al*. (1997) in rice, Chabot *et al*. (1993) in maize. Our results are also in line with similar previous findings. We also found a highly positive correlation (r = 0.79) between root and shoot weight (Table III). Better root development may be due to synergistic relationship of the inoculated bacteria for improving root length and weight by producing growth regulators (Okon, 1985) like IAA and GA (Table I) that along with P fertilizer favored better root development, which enhanced water and nutrient uptake and ultimately helped better shoot development. Single inoculation of either PSB or *Rhizobium* showed no statistical difference over control (Höflich, 2000). However, dual inoculation was the most effective in increasing root/shoot weight. According to Bashan (1998) soil microbial cultures with statistically similar or different functions might express beneficial actions in a soil or rhizosphere.

All inoculations with fertilizer P and dual inoculation without P had significantly greater plant height than other treatments. Single inoculation of *Rhizobium*/PSB, without fertilizer P did not increase plant height over control. Spike length was increased by fertilizer P, dual inoculation and combined application of inoculants + P. Fertilizer P alone and in combinations with inoculations and dual inoculation all significantly increased the grain yield over control as well as single inoculations. These results confirmed the previous findings (Khalid *et al*., 1997; Biswas *et al*., 2000a, b; Hilali *et al*., 2000, 2001), who reported increased plant height and spikelets per spike of various crop plants by microbial inoculation. Plant height and spike length were significantly correlated (r = 0.80 & r = 0.94) with shoot weight and with each other (r = 0.80) (Table III).

Highest grain yield (3.19 g pot^{-1}) was obtained with PSB+P_2O_5 but it was similar to P_2O_5 and combination of

Table I. Some important biochemical features of microbial strains tested during this investigation. Bacterial cultures were grown in respective media incubated for one week. Production of IAA and GA was determined in cell free medium

Bacterial strains	IAA production µg/ml	GA production µg/ml	P solubilization index	Gram reaction
Rhizobium leguminosarum (Thal 8)	65.90	833.7	2.5	Negative
Pseudomonas sp. (54RB)	8.034	1766	4.1	Negative

Table I1. Effect of *Rhizobium* and phosphate solubilizing bacteria (PSB) on agronomic traits, yield components, seed and soil P content, leaf sugar and protein content in wheat

Treatments	Shoot wt. (g pot^{-1})	Root wt. (g pot^{-1})	Plant height (cm)	Spike length (cm)	Grain yield (g pot^{-1})	Seed P content (%)	Soil P content (ppm)	Leaf sugar (µg g^{-1})	Leaf protein (µg g^{-1})
Control (T0)	3.10 c	0.10 c	55.30 b	7.30 c	1.22 b	0.30d	4.10	21.40 c	3767 c
P_2O_5 (T1)	6.22 b	0.30 c	64.00 ab	8.80 bc	2.28 a	0.36c	4.33	70.20 b	3930 bc
Rhizobium (T2)	4.10 c	0.20 c	56.70 b	7.50 c	1.15 b	0.31d	4.67	65.30 b	4474 a
PSB (T3)	4.05 c	0.12 c	55.50 b	7.20 c	1.25 b	0.38c	4.57	25.80 c	3966 bc
Rhizobium + PSB (T4)	6.41 b	0.33 c	68.30 a	9.70 ab	2.85 a	0.29d	4.53	64.50 b	4419 a
Rhizobium + P_2O_5 (T5)	10.3 a	0.68 b	69.30 a	11.00 a	2.31 a	0.45b	4.10	73.70 b	3826 bc
PSB + P_2O_5 (T6)	8.93 a	0.65 b	71.70 a	10.50 ab	3.19 a	0.45b	4.87	66.70 b	4188 ab
Rhizobium + PSB + P_2O_5 (T7)	9.20 a	0.93 a	67.30 a	10.00 ab	2.93 a	0.49a	5.00	94.50 a	4448 a
LSD values	2.075	0.235	9.044	1.551	0.881	0.25	NS	19.31	376.4

Values followed by different letters in a column were significantly different ($P < 0.05$), using Duncan's multiple range test. NS, not significant..PSB, Phosphate Solubilizing Bacteria (*Pseudomonas* sp.) rhizobium (*Rhizobium leguminosarum)*, P_2O_5 @ 0.38 g pot^{-1}

Table III. Correlation effects of *Rhizobium* and phosphate solubilizing bacteria on wheat yield and yield traits (per plant basis)

Variable	Shoot wt	Root wt	Plant height	Spike length	Grain yield	Flag leaf Sugar	Soil P
Root wt.	0.796243**						
Plant height	0.803548**	0.65216**					
Spike length	0.944879**	0.707167**	0.815159**				
Grain yield	0.786934**	0.655576**	0.768131**	0.78742**			
Flag leaf Sugar	0.671061**	0.608289**	0.627023**	0.623612**	0.569902**		
Soil P	0.718864**	0.690239**	0.614421**	0.620678**	0.630866**	0.625122**	
Seed P	0.619352**	0.741055**	0.398551^{NS}	0.471929*	0.34184^{NS}	0.521501**	0.692944**

** = Highly significant at 1% level of probability, * = Significant at 5% level of probability, NS= Non-significant

P_2O_5 and inoculations. *Rhizobium* and PSB alone did not increase the grain yield. Dual inoculation with P_2O_5 had highest seed P content of 0.49%. The *Rhizobium* alone did not increase seed P content. However, PSB alone increase P content as compared to control. Many researchers reported increased seed P content by phosphate solubilizing microorganisms (Kucey, 1987; Mehana & Wahid, 2002; Zaidi *et al.,* 2004), our study revealed the same effect. But dual inoculation without fertilizer could not improve seed P concentration. This may be due to competition of the microbes for P (Azcon-Aguillar *et al.,* 1986). Soil P were not affected by any of the treatment significantly.

Leaf sugar content was significantly increased by *Rhizobium* inoculation and fertilizer treatments. Highest value of leaf sugar content (49.5 µg g^{-1}) was recorded in dual inoculation with fertilizer treated plants. Leaf protein was significantly affected by rhizobial treatments and best treatment in this case was *Rhizobium* along with P_2O_5. This may be due to increased N uptake by a larger root surface area associated with additional root hairs and lateral root development and/or to BNF, either directly by the inoculants strains or indirectly by stimulating BNF activity of the associated rhizosphere community (Ladha *et al*., 1998). Maximum sugar was accumulated in dual inoculated and fertilized plants. Sahin *et al*. (2004) documented increased sugar content in sugar beet by co-inoculation of N-fixing and P solubilizing bacteria.

The present study indicated that single inoculations with *Rhizobium* could not increase grain yield as compared to control. Inoculation with PSB alone increased yield by 2.5% over control (Table II). Dual inoculation of *Rhizobium* and PSB gave yield increases of 29% and 25% with and without fertilizer respectively. Fertilizer P application, however gave yield increases up to 80%. It is concluded that mixed culturing is better than single inoculation. Reduced fertilization (up to 50%) is required with inoculations. Rhizobial and/or PSB inoculaton without fertilization is not effective. *Rhizobium* with non-legumes could act as phosphate solubilizer, hormone producer and to some extent as N-fixer. Addition of *Azospirillum* or other diazotrophs (for N fixation) with present combination would give promising results, which will reduce the N fertilizer use for the profit of farmer and prove environment-friendly.

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